

# Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions

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**Abstract.** 1. Induced plant responses can affect herbivores either directly, by reducing herbivore development, or indirectly, by affecting the performance of natural enemies. Both the direct and indirect impacts of induction on herbivore and parasitoid success were evaluated in a common experimental system, using clonal poplar trees *Populus nigra* (Salicales: Salicaceae), the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), and the gregarious parasitoid *Glyptapanteles flavicoxis* (Marsh) (Hymenoptera: Braconidae).

2. Female parasitoids were attracted to leaf odours from both damaged and undamaged trees, however herbivore-damaged leaves were three times more attractive to wasps than undamaged leaves. Parasitoids were also attracted to herbivore larvae reared on foliage and to larval frass, but they were not attracted to larvae reared on artificial diet.

3. Prior gypsy moth feeding elicited a systemic plant response that retarded the growth rate, feeding, and survival of gypsy moth larvae, however induction also reduced the developmental success of the parasitoid.

4. The mean number of parasitoid progeny emerging from hosts fed foliage from induced trees was 40% less than from uninduced trees. In addition, the proportion of parasitised larvae that survived long enough to issue any parasitoids was lower on foliage from induced trees.

5. A conceptual and analytical model is provided to describe the net impacts of induced plant responses on parasitoids, and implications for tritrophic interactions and biological control of insect pests are discussed.

**Key words.** *Glyptapanteles flavicoxis*, herbivory, induction, *Lymantria dispar*, parasitoid, *Populus nigra*, search behaviour, tritrophic interactions.

## Introduction

Induced plant responses and their effects on herbivores have been demonstrated across a broad range of plant and animal taxa (Karban & Baldwin, 1997). These reactions are often more complex than simple generalised wound responses, and are frequently induced by specific elicitors associated with insects or pathogens (Turlings *et al.*, 1993; Alborn *et al.*, 1997; Korth & Dixon, 1997; Sticher *et al.*, 1997). Responses can vary with plant species or cultivar (Geervliet *et al.*, 1997), the part of the plant being damaged (Coleman *et al.*, 1997), the

herbivore species (Turlings *et al.*, 1998), and herbivore instar (Takabayashi *et al.*, 1995). The effects of induced responses on herbivores have been categorised as direct or indirect depending on their mechanism (Price *et al.*, 1980). Direct induced defences affect herbivore development negatively by reducing the quality of the plant as a food source (Karban & Myers, 1989), while indirect defences enhance performance of the herbivore's natural enemies (Barbosa & Saunders, 1984).

Direct and indirect impacts of induction can alter both behaviour and development. These components have been well described separately, but little is known about the net effects on herbivores and parasitoids, at either the individual fitness or population levels. While most researchers agree that multiple effects are important, the ecological and evolutionary interpretations ascribed to induced plant responses vary, and the

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overall significance of these responses to plant fitness remains unclear (Hunter & Schultz, 1993). Thus, a more inclusive approach could improve interpretations of the ecological and evolutionary significance of inducible responses.

Direct effects of induced plant responses on herbivores have received the most attention. Physiological changes following actual or simulated feeding by herbivores have been shown to reduce the quality of plant tissue for subsequent feeding (e.g. Haukioja & Hanhimäki, 1985; Broadway *et al.*, 1986; Baldwin, 1988; Stout *et al.*, 1998). Such changes can affect either the herbivore eliciting the response (short-term induction), or subsequent herbivore generations or species (long-term induction). The view that induced responses benefit plants directly through impacts on the herbivore is supported by examples of genetic feedback between the plant and the herbivore (e.g. Rossiter *et al.*, 1988; Zangerl & Berenbaum, 1990), the commonality of defences against herbivores and pathogens (Levin, 1976), and the complication that relying on natural enemy abundance can be risky and unpredictable (Karban & Baldwin, 1997).

Early interpretations of how induced responses could benefit plants indirectly emphasised the possibility that prolonged herbivore development could increase exposure to natural enemies (Price *et al.*, 1980; Schultz, 1983). Although this seems likely, few studies have actually documented such impacts (Bergelson & Lawton, 1988; Haggström & Larsson, 1995; Benrey & Denno, 1997; Bernays, 1997). More recent studies have emphasised the attractiveness of induced plant allelochemicals to parasitoids and predators (Vet & Dicke, 1992; Agrawal, 1999). Host habitat location by parasitoids is greatly enhanced by *infochemicals* released by plants following herbivore feeding (e.g. Turlings *et al.*, 1991, 1998; Dicke *et al.*, 1993; Rose *et al.*, 1998). The composition and timing of these volatile emissions suggest an active role in plant defence and a coevolutionary relationship between plants and parasitoids (Mattiacci *et al.*, 1994; Turlings *et al.*, 1995; Pere & Tumlinson, 1997; Vinson, 1998). Support for this interpretation includes the mortality exerted by natural enemies in contrast to mere reductions in herbivore feeding, development rate, and fecundity commonly seen with direct effects. Moreover, parasitoids exert immediate impacts on herbivores in contrast to the delays that typically accompany the direct effects of induced responses. Such delayed effects of induction do not necessarily reduce feeding by the inciting herbivore, but rather reduce herbivore population growth over time. Moreover, delayed responses may allow insect herbivores time to acclimate physiologically and thereby negate induced responses (Krause & Raffa, 1992).

A third factor to consider is whether induced responses affect parasitoid developmental success. Plant allelochemicals can reduce parasitoid success directly, or indirectly due to retarded host development (Campbell & Duffy, 1979; Barbosa *et al.*, 1991; Werren *et al.*, 1992; Kruse & Raffa, 1997; Reitz & Trumble, 1997; Roth *et al.*, 1997). In general, as the suitability of a host plant for herbivore development declines, so does the developmental performance of associated parasitoids. Decreased suitability of plants due to induction may have similar effects on parasitoids.

A thorough understanding of tritrophic interactions should incorporate the potentially opposing effects of inducible plant responses on the behavioural and developmental success of parasitoids and herbivores. Such information could provide further insight into the role of induction on insect population dynamics, plant–insect coevolution, and the efficacy of biological control programmes (Thaler, 1999).

The experimental system for this study consisted of *Populus nigra* (Salicales: Salicaceae), *Lymantria dispar* L. (Lepidoptera: Lymantriidae), and *Glyptapanteles flavicoxis* (Marsh) (Hymenoptera: Braconidae). *Populus* trees can be propagated vegetatively, which allows for high genetic control in induction studies (Havill & Raffa, 1999). Phenolic glycosides, and to a lesser extent tannins, comprise the major defensive compounds in *Populus* against Lepidoptera (Bryant *et al.*, 1987; Lindroth & Hemming, 1990; Lindroth & Bloomer, 1991), and chemical induction is an important component in its resistance to herbivory (Palo, 1984; Mattson & Palmer, 1988; Clausen *et al.*, 1989; Bradshaw *et al.*, 1991; Reichenbacher *et al.*, 1996; Robison & Raffa, 1997). The gypsy moth *L. dispar* is a polyphagous folivore, which shows highest preference for *Quercus* spp. and *Populus* spp. (Liebhold *et al.*, 1995). Previous work has shown behavioural preference by *L. dispar* for damaged over induced *Populus* trees, and that the extent of this effect varies among clones (Havill & Raffa, 1999). *Glyptapanteles flavicoxis* is a gregarious endoparasitoid that oviposits in late-instar gypsy moth larvae. *Glyptapanteles flavicoxis* clutch size has been shown to increase with host age and decrease with parasitoid age (Hu *et al.*, 1986), but the effects of larval feeding substrate on *G. flavicoxis* development have not been tested. Several hymenopteran parasitoids of *L. dispar* have been shown to be affected by tree species (e.g. Werren *et al.*, 1992), but there are no previous reports of long-range attraction to wounded trees.

## Materials and methods

### *Plant and insect culture*

Trees of poplar clone NC5271 (*P. nigra* 'Charkowiensis' × *P. nigra* 'Caudina') were established from sources growing in a common garden at the University of Wisconsin West Madison Agricultural Research Station. Previous work has demonstrated that this clone is highly preferred by *L. dispar*, is inducible, and shows low between-tree variation in plant quality to herbivores (Robison & Raffa, 1997; Havill & Raffa, 1999). Dormant hardwood cuttings (20 cm long) were dipped in Hormex no. 8 rooting powder (0.8% Indole-3-Butyric Acid; Brooker Chemical, North Hollywood, California) and planted in saturated Fafard no. 2 potting soil (Fafard Inc., Agawam, Massachusetts) in 15.12-l plastic pots. Trees were fertilised with 15 g/plant Osmocote 19-6-12 slow release fertiliser (Sierra Chemical Co., Milpitas, California) plus micronutrients and flood irrigated daily. Trees were grown at the University of Wisconsin-Madison Biotron facility in a climate-controlled greenhouse at 24 °C, with a LD 16:8 h regime. Because larval performance can vary sig-

nificantly according to leaf age in *Populus*, all experiments were standardised for leaf position. The most apical fully unfolded leaf was designated as leaf 1 and numbering continued sequentially down the stem. To avoid leaf-age effects (Bingaman & Hart, 1993; Robison & Raffa, 1997), only leaves from positions 5–9 were fed to gypsy moth larvae in all experiments. Leaves within this range have been shown to have similar suitability to insect herbivores (Bingaman & Hart, 1993; Robison & Raffa, 1997), which is subsequently confirmed for this system, so different leaves within this range should not affect herbivores or parasitoids differently (see Results). Trees for all experiments were  $\approx 50$  days old and had between 20 and 26 leaves (0.88–1.09 m tall).

For all experiments, *L. dispar* larvae were reared from egg masses of strain NJSS obtained from the Otis Methods Development Center (USDA, APHIS, Otis AFB, Massachusetts). Larvae were reared using previously described methods (Kruse & Raffa, 1997). Before hatching, pre-chilled egg masses were surface sterilised in a sodium hypochlorite solution (2060 ml dH<sub>2</sub>O, 21 ml polyoxyethylene sorbitan monooleate, and 40 ml bleach) for 5 min, rinsed three times with dH<sub>2</sub>O, and allowed to dry. Egg masses were then placed in 16 fluid oz unwaxed paper cups and reared under a LD 16:8 h photoperiod at 24 °C and 50–70% RH in an environmental growth chamber. On hatching, the larvae were provided with poplar leaves, or two 2 cm<sup>3</sup> cubes of artificial diet (ICN Biomedicals, Aurora, Ohio), which were replaced every 2–3 days as needed.

Adult *G. flavicoxis* were obtained from the USDA ARS Beneficial Insects Introduction Laboratory, Newark, Delaware. Wasps were maintained in culture on gypsy moth larvae reared on *Populus* foliage and kept under a LD 18:6 photoperiod at 24 °C and 50–70% RH, using modified methods of Hu *et al.* (1986). Newly emerged wasp pupae were placed in 240-ml plastic cups with honey and water so that adult wasps could feed upon emergence. Wasps were allowed to mate for 24 h, after which females were removed with an aspirator, placed in plastic cups, provided with honey and water, and kept in a growth chamber at 15 °C until needed for experiments or culture maintenance.

#### Behavioural assays

A two-way olfactometer was developed to evaluate the effects of volatile odours on *G. flavicoxis* females (Fig. 1). The olfactometer arena consisted of a 5-cm long, 6-cm diameter clear glass cylinder, open at the bottom and covered at the top with fine nylon mesh. This arena rested on a 9-cm diameter glass base plate pierced with two 1-cm diameter holes, 4 cm apart. The base plate was made of frosted glass to obscure any visual cues from below that might affect parasitoid choice. Odour sources were placed directly under the holes in the base plate in 125-ml Erlenmeyer flasks with side tubulation. A tank of compressed medical air provided airflow for the olfactometer. Air was passed through a flask containing distilled water to provide humidity. The airflow was divided in two,

directed through the arms of the odour source flasks and through the holes in the base plate. This resulted in a steady stream of air extending up opposite sides of the interior of the cylindrical arena. The airflow through each side of the olfactometer was held at 500 ml min<sup>-1</sup>, and monitored with a flowmeter. A single light source consisting of a 15-W incandescent lamp directed at the ceiling of the bioassay room provided even, diffused lighting. The temperature in the bioassay room was maintained at 23–25 °C. Preliminary results indicated that this olfactometer system has some significant advantages over the more traditional y-tube. Wasps can make longer flight attempts, the small holes leading to the odour sources eliminate some of the error from random wandering in y-tubes, and results are more consistent (Havill, 1998).

Parasitoid host searching behaviour was tested using the following six odour sources: (1) a blank consisting of an empty odour source flask; (2) three gypsy moth larvae reared on artificial diet since hatch; (3) three gypsy moth larvae reared on poplar foliage since hatch; (4) 1 ml of frass from gypsy moths feeding on poplar leaves for 24 h; (5) an undamaged leaf that was removed from a tree 24 h before initiation of the bioassay and rinsed briefly with distilled water; (6) a damaged leaf that had been fed on by three gypsy moth larvae for 24 h. Immediately before the assay, the larvae were removed from the leaf, any frass or silk was carefully cleaned off with a soft brush and the leaf was rinsed briefly with distilled water. Treatments 2–6 were assayed against the blank control; treatments 5 and 6 were tested against each other.

All plant-derived odour sources were prepared from poplar leaves collected from leaf position number 5 and placed in water tubes containing distilled water. All gypsy moths used as odour sources were randomly selected fourth-instar larvae fed poplar foliage since hatch unless otherwise stated.

All olfactometer tests were performed 6–10 h after the beginning of the photophase. Adult female wasps, 1–3 days old, were introduced individually into the olfactometer arena by aspiration through a small slit in the screen covering the top. All females were naive, with no previous oviposition experience or adult contact with plants. Wasps were able to walk freely in the olfactometer arena and make short flight attempts. A choice was scored when a wasp moved upwind and entered the neck of either odour source flask, at which time it was removed quickly before encountering the odour source itself. If no choice was made after 5 min, the wasp was removed and considered a *no response*. Each wasp was used only once. In order to remove any spatial bias, the positions of odour source flasks were exchanged after each individual was tested. After a series of five wasps, new odour sources were provided, and the entire apparatus was cleaned thoroughly with soap and rinsed with acetone. Assays were repeated over several days with 20–40 wasps tested per day.

The olfactometer choice assays were analysed using a chi-square test (d.f.<sub>1</sub>,  $P=0.05$ ) to determine differences between observed and expected (1:1) values for each combination of odour sources.

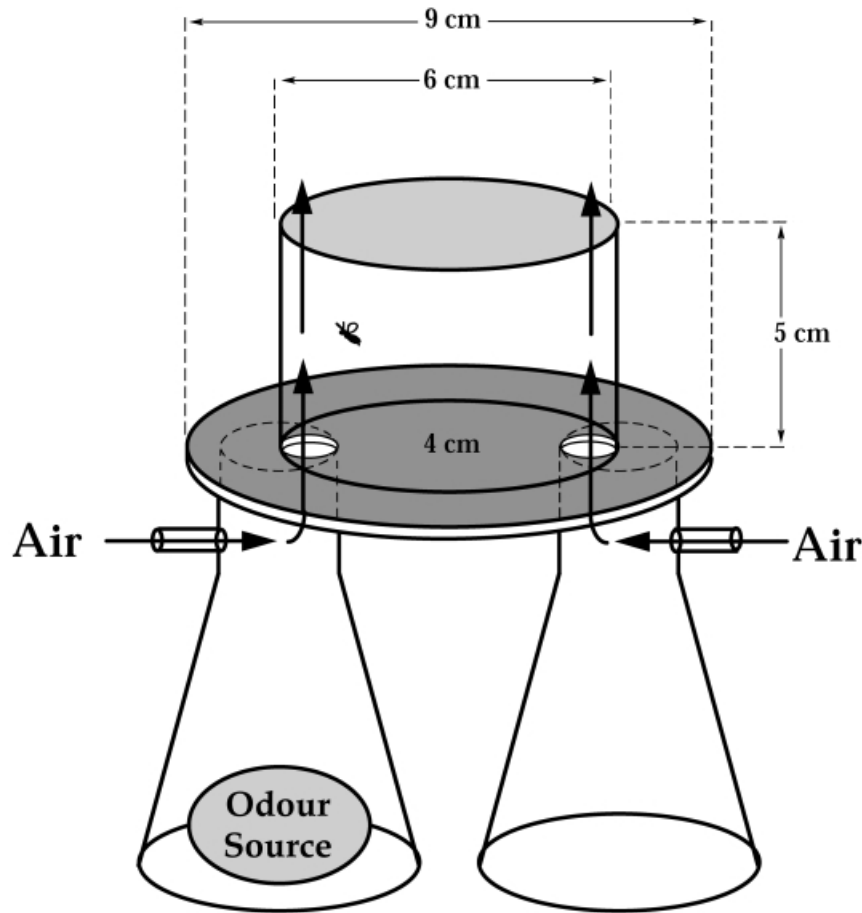


Fig. 1. Schematic drawing of two-choice olfactometer for evaluating effects of plant and host odours on behaviour of parasitoids.

#### Development assays

No-choice assays were performed to determine how induced tree responses affect the development and reproductive success of *L. dispar* and *G. flavicoxis*. Newly hatched gypsy moth larvae were assigned randomly to a diet of leaves from either induced or uninduced trees. Induced trees were damaged by enclosing six fifth-instar gypsy moth larvae on leaf numbers 11–16 within white nylon screen sleeves. Larvae were allowed to feed for 24 h, then removed. Upper, undamaged leaves (positions 6–9) were collected 72 h following initiation of induction, placed in water picks with distilled water, and fed to larvae. Larvae assigned to the uninduced treatment were fed leaves from undamaged trees.

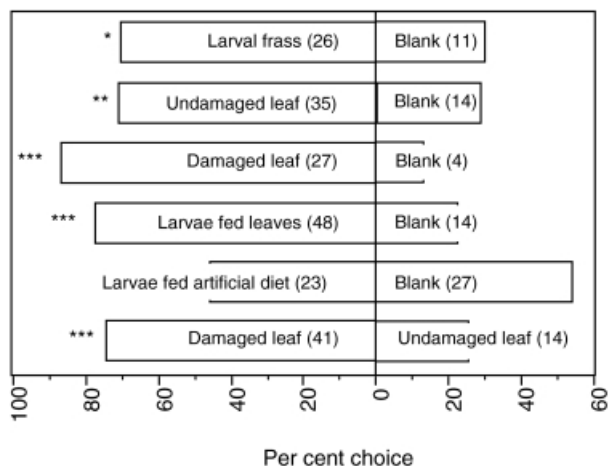
All trees that provided leaves for a particular replicate were grown as a group in the greenhouse. Twenty groups of eight trees were assigned randomly to receive either the induced or uninduced treatment. Every 6 days, gypsy moths were given fresh leaves from a new tree. Trees within a group were planted at 6-day intervals to ensure that no tree was sampled more than once and that all larvae received leaves from 50-day-old trees at each feeding. To improve the power of the experimental design, each full replicate initially consisted of

six gypsy moth larvae (subsamples). Three larvae were reared together in a cup and fed leaves from positions 6–7; three were reared in a separate cup and fed leaves from positions 8–9. No leaf age effect was expected for leaves within the range of positions 6–9.

Upon moulting to the third stadium, each gypsy moth larva was weighed and introduced into an arena with two or three mated *G. flavicoxis* females for parasitisation. Each larva was attacked by only one female and the oviposition event was observed. A maximum of five larvae was parasitised by each wasp.

After parasitisation, larvae were fed foliage appropriate to their treatment group. Larvae were monitored daily for the presence of emerged *G. flavicoxis* pupae, gypsy moth mortality, or pupation. Parasitoid sex, larval development time, and size were recorded. Parasitoid size, which can be indicative of fecundity, searching efficiency, longevity, and mating success (Godfray, 1994; Ellers *et al.*, 1998), was measured under a microscope fitted with an ocular micrometer using hind tibia length as an index of overall size.

Effects of induction on gypsy moth survival were analysed as a split-plot ANOVA (PROC GLM; SAS Institute, 1990), with induction treatment as the whole-plot error and leaf position as



**Fig. 2.** Response of female *Glyptapanteles flavicoxis* in two-choice olfactometer assay. Each set of bars shows the per cent of responding wasps that chose a particular odour within a combination. Actual number of wasps responding is shown in parenthesis, and asterisks denote a significant preference for a particular odour source (chi-square d.f.<sub>1</sub>: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). The numbers of nonresponding wasps were as follows: frass vs. blank, 22; undamaged leaf vs. blank, 35; damaged leaf vs. blank, 12; larvae fed leaves vs. blank, 12; larvae fed diet vs. blank, 7; damaged vs. undamaged leaf, 16.

the subplot error. The effects of induction on gypsy moth weight and development time, the number of parasitoid progeny per host, and parasitoid development time and sex ratio were analysed as above, but data from individual gypsy moths provided additional subsampling error. Parasitoid tibia lengths were also analysed as above but with subsampling errors for both individual gypsy moths and individual wasps. Residuals were examined (PROC UNIVARIATE; SAS Institute, 1990) for normality and log or arcsin-square root transformations were performed where needed to achieve homogeneity of variance.

## Results

### *Behavioural response to volatiles*

Adult female *G. flavicoxis* showed a clear attraction to plant-derived odour sources (Fig. 2). Parasitoids were attracted strongly to larval frass ( $\chi^2 = 6.08$ ,  $P < 0.05$ ), undamaged poplar leaves ( $\chi^2 = 9.0$ ,  $P < 0.01$ ), and damaged poplar leaves ( $\chi^2 = 17.05$ ,  $P < 0.001$ ) when tested against blank controls. Attraction relative to controls was greater for damaged than for undamaged leaves. Wasps were attracted to larvae reared on poplar leaves when tested against a blank ( $\chi^2 = 18.65$ ,  $P < 0.001$ ), but not to larvae raised on artificial diet ( $\chi^2 = 0.32$ ,  $P = \text{NS}$ ). Given a choice between damaged and undamaged leaves, response to damaged leaves was almost three times higher ( $\chi^2 = 13.25$ ,  $P < 0.001$ ).

### *Development assays*

Effects of host plant induction on gypsy moth development. Larval development from hatch to moulting into the third instar was affected significantly by a diet of induced foliage (Table 1). Larvae that were reared on leaves from herbivore-damaged trees required approximately 2.5 days longer to develop to the third stadium than larvae fed leaves from undamaged trees ( $P < 0.01$ ). In addition, larvae fed leaves from uninduced trees were 33% heavier than larvae fed leaves from induced trees ( $P < 0.01$ ). The proportion of parasitised larvae that survived long enough to issue parasitoids was 24% higher for larvae fed leaves from uninduced trees than for larvae fed leaves from induced trees ( $P < 0.01$ ). There were no significant leaf position effects or interactions ( $P > 0.05$ ) between induction treatment and leaf position for any host development parameter, thus validating the similarity of the leaves selected for bioassay.

*Effects of host plant induction on parasitoid development.* Significantly fewer parasitoids emerged from gypsy moth larvae feeding on leaves from damaged than undamaged trees ( $P < 0.05$ ) (Table 2). The hind tibiae of male wasps that emerged from hosts fed induced foliage were significantly shorter ( $P < 0.05$ ) than those from controls (Table 2). Female tibia length ( $P = \text{NS}$ ), parasitoid larval development time ( $P = \text{NS}$ ), and parasitoid sex ratios ( $P = \text{NS}$ ) were not affected significantly by host plant induction, although the trend with respect to fitness was for these parameters to be enhanced among wasps that emerged from larvae feeding on uninduced trees (Table 2). There were no significant leaf position effects or interactions between induction treatment and leaf position ( $P > 0.05$ ) for any parasitoid development parameter.

## Discussion

These results demonstrate that induced responses can benefit plants through effects on both herbivores and natural enemies, but that individual plant responses can also exert detrimental effects on natural enemy populations. These relationships are governed by both behavioural and developmental mechanisms that result in opposing effects on parasitoid and herbivore success.

Female parasitoids were attracted to odour sources associated with host plants, especially host plants damaged by the herbivore. Leaves that were damaged by larval gypsy moth feeding attracted nearly three times as many wasps as did undamaged leaves. Cues associated solely with the herbivore, i.e. gypsy moth larvae reared on artificial diet, were not attractive to wasps, whereas larvae that had prolonged contact with damaged plants were attractive. These results agree with observations from other tritrophic systems (e.g. Dicke *et al.*, 1993).

Induction directly reduced the performance of early-instar gypsy moth larvae. By the third stadium, larvae reared on induced foliage were 33% smaller and required 2.5 more days to develop. Prior feeding also induces changes in this poplar

**Table 1.** Effects of host plant induction on larval gypsy moth development. Means  $\pm$  SE followed by different letters are significantly different ( $P < 0.05$ ).

Treatment	<i>n</i>	Development time (days)*	<i>n</i>	Weight at third instar (mg)†	<i>n</i>	Per cent survival of parasitised larvae‡
Control	19	17.65 $\pm$ 0.42 a	19	36.7 $\pm$ 2.2 a	20	67.92 $\pm$ 5.48 a
Induced	19	20.18 $\pm$ 0.63 b	19	27.5 $\pm$ 2.2 b	19	43.87 $\pm$ 7.32 b
<i>F</i> ; d.f.		10.99; 1,36		9.53; 1,36		7.79; 1,37
<i>P</i>		0.002		0.004		0.008

\*Development time from hatch to moulting into the third instar.

†Weights were taken at the moult to the third instar and were log-transformed prior to analysis. Actual values are presented.

‡Survival after parasitisation. Values were arcsin-square root transformed prior to analysis. Actual values are presented.

**Table 2.** Effects of host plant induction on *Glyptapanteles flavicoxis* development. Means  $\pm$  SE followed by different letters are significantly different ( $P < 0.05$ ).

Treatment	<i>n</i>	Parasitoid progeny per host	<i>n</i>	Male tibia length (mm)	<i>n</i>	Female tibia length (mm)	<i>n</i>	Larval development time (days)	<i>n</i>	Sex ratio (% female)*
Control	19	14.71 $\pm$ 1.48 a	18	0.811 $\pm$ 0.008 a	17	0.904 $\pm$ 0.008 a	19	18.55 $\pm$ 0.59 a	18	37.24 $\pm$ 3.95 a
Induced	14	8.97 $\pm$ 1.22 b	13	0.783 $\pm$ 0.012 b	13	0.898 $\pm$ 0.017 a	14	18.93 $\pm$ 1.01 a	14	29.49 $\pm$ 7.64 a
<i>F</i> ; d.f.		5.26; 1,31		5.54; 1,29		0.75; 1,28		0.17; 1,31		2.30; 1,30
<i>P</i>		0.03		0.03		0.39		0.68		0.14

\*Sex ratios were arcsin-square root transformed prior to analysis. Actual values are presented.

clone that significantly reduce gypsy moth feeding on damaged leaves (Havill & Raffa, 1999).

Induction also reduced the reproductive success of the herbivore's parasitoid. The mean number of wasp progeny emerging from *L. dispar* feeding on induced foliage was 40% less than from hosts feeding on control foliage. In addition, more host larvae reared on leaves from induced than control trees died before parasitoid emergence. These results show that induction can reduce parasitoid fitness, but the extent to which this is due to direct toxic effects of induced allelochemicals or decreased host quality due to growth inhibition could not be determined. The reduced size of males emerging from larvae fed on induced trees could have additional population-level effects. Smaller male wasps of other species can have decreased insemination capacity, shorter lifespans, and lower mating frequency (van den Assem *et al.*, 1989). These results also suggest that use of larger sample sizes may have determined that induced plant responses reduced female wasp size and female sex ratios (Table 2).

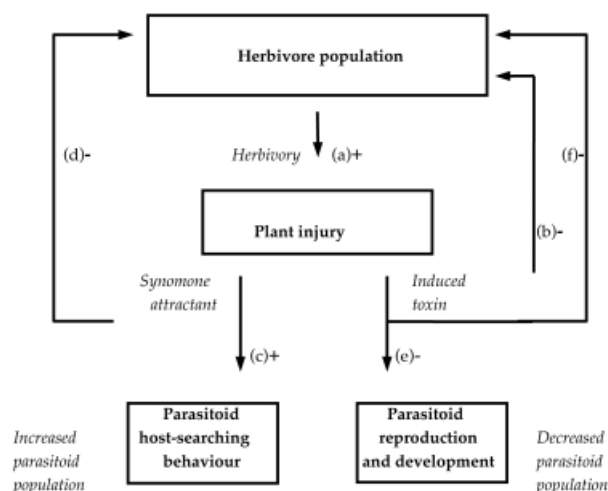
A conceptual diagram showing the multiple and opposing impacts of induced plant responses on herbivore and parasitoid development and insect population dynamics is shown in Fig. 3 (see figure caption for definitions). Each of these relationships has been demonstrated within the system described here, and portions have been described in many other systems. Increased herbivore populations result in increased feeding, which in turn elicits quantitative and qualitative changes in host plant chemistry (Fig. 3a) (Robison & Raffa, 1997). These changes reduce herbivore feeding, development, and reproduction

(Table 1, Fig. 3b) (Havill & Raffa, 1999), which reduce herbivore populations and plant injury. Concurrently, volatiles associated with wounding increase attraction by parasitoids (Fig. 2), which further reduce herbivore populations and provide immediate protection to host plants (Fig. 3c,d). Negative impacts on parasitoid development (Fig. 3e,f) can also occur (Table 2). If this model is expanded to include systems in which allelochemicals affect the virulence of entomopathogens (Hunter & Schultz, 1993; Hoover *et al.*, 1998), antagonistic interactions could amplify the positive feedback of induced plant responses on herbivore populations (e.g. Appel & Schultz, 1994), whereas synergistic interactions could amplify negative feedback (e.g. Hwang *et al.*, 1995). The prevalence of both positive and negative effects, with both rapid and delayed feedback, could contribute to the herbivore population oscillations that have been proposed to arise from plant induction (Haukioja & Hanhimäki, 1985; Lundberg *et al.*, 1994).

Quantifying the net impacts of plant induction on parasitoids (Fig. 3c vs. 3e) poses a significant challenge that requires detailed experiments at different scales, however a general working model can be formulated in which the overall effect can be stated as:

$$\text{Net impact of induction} = (l_i \times m_i \times a_i) / (l_u \times m_u \times a_u)$$

where *l* refers to the proportion of parasitised hosts that survive long enough to issue parasitoids, *m* refers to the average number of female parasitoids, and *a* refers to parasitoid search efficiency. The subscripts *i* and *u* refer to *induced* and



**Fig. 3.** Multiple effects of induced responses to herbivory in tritrophic interactions. + indicates that an increase in one parameter (within the previous box) causes an increase in a second parameter (within the next box), i.e. a direct relationship. – indicates that an increase in one parameter causes a decrease in a second parameter, i.e. an inverse relationship. Italicised descriptors indicate processes by which these relationships might be governed. Note that either a series of + relationships or an even number of – relationships (e.g. a-e-f) generates a positive feedback loop, whereas odd numbers of – relationships coupled with + relationships (e.g. a-c-d) generate negative feedback.

uninduced trees. Together, the parameters  $l$  and  $m$  indicate parasitoid developmental success. In the system described here, parasitoids that oviposit in larvae on induced foliage produce only 0.31 times as many females as those that oviposit in larvae on uninduced foliage (Table 1 and 2). Incorporating relative searching efficiency into the above model is more difficult because olfactometers are necessarily artificial arenas. The best estimate that can be provided from this experimental system is from the data in Fig. 2. Search efficiency for this model is calculated as the number of parasitoids that chose either an induced or an uninduced tree in an olfactometer experiment, divided by the total number of trials for that experiment. If the comparisons of leaf tissues vs. blanks are used to calculate search efficiency (27/31 for induced; 35/49 for uninduced), the net impact of induction remains substantially below 1.0, but rises to 0.38 (0.46 if nonresponding parasitoids are included). If damaged vs. undamaged leaves (which could simulate parasitoid choice in a forest that includes both induced and uninduced trees) is used as the basis for comparison (41/55 for induced; 14/55 for uninduced), the overall performance ratio rises to 0.91. This value indicates a strong trade-off between direct and indirect effects of induction on the parasitoid but little net impact on the plant. Field experiments under both choice and no-choice conditions are needed to test this general model fully. Effects of plant induction on host searching efficiency by parasitoids probably vary with plant and insect species, level of feeding, community structure, and weather. Moreover, if wounding elicits between-

plant communication, as has been proposed in some systems (Baldwin & Schultz, 1983; Rhoades, 1983; Farmer & Ryan, 1990), any improved parasitoid efficiency due to induction could be diluted, thus reducing some of the indirect benefits of plant induction.

These results also have implications for the biological control of insect pests. *Lymantria dispar* is an invasive species and is considered the most destructive defoliator of deciduous trees in North America (Liebhold *et al.*, 1995). It is expanding its range to new forest regions despite extensive control efforts. Despite suggestions that manipulating stimuli from host plants could improve pest management (Greenblatt & Lewis, 1983; Roland *et al.*, 1995; Bottrell *et al.*, 1998), few data are available regarding induced infochemicals from trees. These results suggest that previous proposals to exploit plant infochemicals for agricultural crop production hold equivalent promise in tree plantations and forest ecosystems, however adverse effects on biological control by parasitoids (Fig. 2) and predators should also be considered. An understanding of the multiple effects of plant induction on herbivores and parasitoids could help to guide the integration of silviculture, resistance breeding, and biological control.

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